METHODS AND COMPOSITIONS FOR THE AMPLIFICATION OF MUTATIONS IN THE DIAGNOSIS OF CYSTIC FIBROSIS

Field of the Invention

[0001] The present invention relates to nucleotide sequences useful as primers for amplifying portions of the cystic fibrosis transmembrane regulator (CFTR) gene where cystic fibrosis (CF) mutations are known to arise, and use of the amplified sequence to identify the presence or absence of CF mutant sequences in a biological sample.

Background of the Invention

[0002] The following description of the background of the invention is provided simply as an aid in understanding the invention and is not admitted to describe or constitute prior art to the invention.

[0003] Cystic fibrosis (CF) is the most common severe autosomal recessive genetic disorder in the Caucasian population. It affects approximately 1 in 2,500 live births in North America (Boat et al, The Metabolic Basis of Inherited Disease, 6th ed, pp 2649-2680, McGraw Hill, NY (1989)). Approximately 1 in 25 persons are carriers of the disease. The responsible gene has been localized to a 250,000 base pair genomic sequence present on the long arm of chromosome 7. This sequence encodes a membrane-associated protein called the "cystic fibrosis transmembrane regulator" (or "CFTR"). There are greater than 1000 different mutations in the CFTR gene, having varying frequencies of occurrence in the population, presently reported to the Cystic Fibrosis Genetic Analysis Consortium. These mutations exist in both the coding regions (*e.g.*, ΔF508, a mutation found on about 70% of CF alleles, represents a deletion of a phenylalanine at residue 508) and the non-coding regions (*e.g.*, the 5T, 7T, and 9T mutations correspond to a sequence of 5, 7, or 9 thymidine bases located at the splice branch/acceptor site of intron 8) of the CFTR gene.

[0004] The major symptoms of cystic fibrosis include chronic pulmonary disease, pancreatic exocrine insufficiency, and elevated sweat electrolyte levels. The symptoms are consistent with cystic fibrosis being an exocrine disorder. Although recent advances have been made in the

analysis of ion transport across the apical membrane of the epithelium of CF patient cells, it is not clear that the abnormal regulation of chloride channels represents the primary defect in the disease.

Summary of the Invention

[0005] The present invention provides compositions and methods for amplifying CFTR nucleic acid sequences and for using such amplified sequence to identify the presence of absence of CF mutations in the CFTR gene. In particular, nucleic acid primers are provided herein for amplifying segments of the CFTR gene that are known to contain mutant cystic fibrosis (CF) nucleic acid sequence. These primers therefore enable the construction of assays that utilize amplification methods, preferably the polymerase chain reaction (PCR), to amplify the nucleic acid sequences in a biological sample for detection of mutant gene sequence. The present invention therefore further discloses methods for detecting individual mutant CF sequence in the amplfied product(s).

[0006] In a first aspect, the present invention provides one or more substantially pure nucleic acid sequences, and/or complementary sequences thereof, that can be used as primers to amplify segments of the CFTR gene where CF mutant nucleic acid sequences are known to arise.

[0007] The primers of the present invention hybridize to a CFTR coding sequence or a CFTR non-coding sequence, or to a complement thereof. Suitable primers are capable of hybridizing to coding or non-coding CFTR sequence under stringent conditions. The primers may be complementary to CF predetermined nucleic acid sequences that are associated with cystic fibrosis or may flank one or more such sequences. Preferred primers are those that flank mutant CF sequences. Primers may be labeled with any of a variety of detectable agents such as radioisotopes, dyes, fluorescent molecules, haptens or ligands (e.g., biotin), and the like. In a preferred approach, the primer are labeled with biotin. The biotin label is preferably attached to the 5' end of the primer.

[0008] By "predetermined sequence" is meant a nucleic acid sequence that is known to be associated with cystic fibrosis. Predetermined sequence that is known to be associated with cystic fibrosis includes mutant CF nucleotide sequence.

[0009] By "mutant CF nucleic acid sequence," "CF mutant sequences," or "genotype for cystic fibrosis" is meant one or more CFTR nucleic acid sequences that are associated or

correlated with cystic fibrosis. These mutant CF sequences may be correlated with a carrier state, or with a person afflicted with CF. The nucleic acid sequences are preferably DNA sequences, and are preferably genomic DNA sequences; however, RNA sequences such as mRNA or hnRNA may also contain nucleic acid sequences that are associated with cystic fibrosis. Mutations in the cystic fibrosis gene are described, for example, in U.S. Patent 5,981,178 to Tsui et al., including mutations in the cystic fibrosis gene at amino acid positions 85, 148, 178, 455, 493, 507, 542, 549, 551, 560, 563, 574, 1077, and 1092, among others. Also disclosed are mutant DNA at nucleotide sequence positions, 621+1, 711+1, 1717-1 and 3659, which encode mutant CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) polypeptide. Preferred sequences known to be associated with CF are described hereinafter, *e.g.*, in Table 1.

[0010] By "carrier state" is meant a person who contains one CFTR allele that is a mutant CF nucleic acid sequence, but a second allele that is not a mutant CF nucleic acid sequence. CF is an "autosomal recessive" disease, meaning that a mutation produces little or no phenotypic effect when present in a heterozygous condition with a non-disease related allele, but produces a "disease state" when a person is homozygous, i.e., both CFTR alleles are mutant CF nucleic acid sequences.

[0011] By "primer" is meant a sequence of nucleic acid, preferably DNA, that hybridizes to a substantially complementary target sequence and is recognized by DNA polymerase to begin DNA replication.

[0012] By "substantially complementary" is meant that two sequences hybridize under stringent hybridization conditions. The skilled artisan will understand that substantially complementary sequences need not hybridize along their entire length. In particular, substantially complementary sequences comprise a contiguous sequence of bases that do not hybridize to a target sequence, positioned 3' or 5' to a contiguous sequence of bases that hybridize under stringent hybridization conditions to a target sequence.

[0013] By "flanking" is meant that a primer hybridizes to a target nucleic acid adjoining a region of interest sought to be amplified on the target. The skilled artisan will understand that preferred primers are pairs of primers that hybridize 3' from a region of interest, one on each strand of a target double stranded DNA molecule, such that nucleotides may be add to the 3' end of the primer by a suitable DNA polymerase. Primers that flank mutant CF sequences do not

actually anneal to the mutant sequence but rather anneal to sequence that adjoins the mutant sequence.

[0014] By "isolated" a nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany such nucleic acid. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, oligonucleotides, and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

[0015] By "substantially pure" a nucleic acid, represents more than 50% of the nucleic acid in a sample. The nucleic acid sample may exist in solution or as a dry preparation.

[0016] By "complement" is meant the complementary sequence to a nucleic acid according to standard Watson/Crick pairing rules. For example, a sequence (SEQ ID NO: 1) 5'-GCGGTCCCAAAAG-3' has the complement (SEQ ID NO: 2) 5'-CTTTTGGGACCGC-3'. A complement sequence can also be a sequence of RNA complementary to the DNA sequence or its complement sequence, and can also be a cDNA.

[0017] By "coding sequence" is meant a sequence of a nucleic acid or its complement, or a part thereof, that can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. Coding sequences include exons in a genomic DNA or immature primary RNA transcripts, which are joined together by the cell's biochemical machinery to provide a mature mRNA. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[0018] By "non-coding sequence" is meant a sequence of a nucleic acid or its complement, or a part thereof, that is not transcribed into amino acid *in vivo*, or where tRNA does not interact to place or attempt to place an amino acid. Non-coding sequences include both intron sequences in genomic DNA or immature primary RNA transcripts, and gene-associated sequences such as promoters, enhancers, silencers, *etc.*

[0019] In preferred embodiments the substantially pure nucleic acid sequence(s) is(are) a DNA (or RNA equivalent) that is any of the following:

SEQ ID NO: 3

5'- GCGGTCCCAAAAGGGTCAGTTGTAGGAAGTCACCAAAG -3' (g4e1F)

SEQ ID NO: 4

5'- GCGGTCCCAAAAGGGTCAGTCGATACAGAATATATGTGCC -3' (g4e2R)

SEQ ID NO: 5

5'- GCGGTCCCAAAAGGGTCAGTGAATCATTCAGTGGGTATAAGCAG -3' (g19i2F)

SEQ ID NO: 6

5'- GCGGTCCCAAAAGGGTCAGTCTTCAATGCACCTCCTCCC -3' (q19i3R)

SEQ ID NO: 7

5'- GCGGTCCCAAAAGGGTCAGTTAGATACTTCAATAGCTCAGCC -3' (g7e1F)

SEQ ID NO: 8

5'- GCGGTCCCAAAAGGGTCAGTGGTACATTACCTGTATTTTGTTT -3' (g7e2R)

SEQ ID NO: 9

5'- GCGGTCCCAAAAGGGTCAGTGTGAATCGATGTGGTGACCA -3' (s12e1F)

SEQ ID NO: 10

5'- GCGGTCCCAAAAGGGTCAGTCTGGTTTAGCATGAGGCGGT -3' (s12e1R)

SEQ ID NO: 11

5'- GCGGTCCCAAAAGGGTCAGTTTGGTTGTGCTGTGGCTCCT -3' (g14be1F)

SEQ ID NO: 12

5'- GCGGTCCCAAAAGGGTCAGTACAATACATACAAACATAGTGG -3' (g14e2R)

SEQ ID NO: 13

5'- GCGGTCCCAAAAGGGTCAGTGAAAGTATTTATTTTTTCTGGAAC -3' (q21e1F)

SEQ ID NO: 14

5'- GCGGTCCCAAAAGGGTCAGTGTGTGTAGAATGATGTCAGCTAT -3' (q21e2R)

SEQ ID NO: 15

5'- GCGGTCCCAAAAGGGTCAGTCAGATTGAGCATACTAAAAGTG -3' (g11e1F)

SEQ ID NO: 16

5'- GCGGTCCCAAAAGGGTCAGTTACATGAATGACATTTACAGCA -3' (g11e2R)

SEQ ID NO: 17

5'- GCGGTCCCAAAAGGGTCAGTAAGAACTGGATCAGGGAAGA -3' (g20e1F)

SEQ ID NO: 18

5'- GCGGTCCCAAAAGGGTCAGTTCCTTTTGCTCACCTGTGGT -3' (g20e2R)

SEQ ID NO: 19

5'- GCGGTCCCAAAAGGGTCAGTGGTCCCACTTTTTATTCTTTTGC -3' (q3e2F)

SEQ ID NO: 20

5'- GCGGTCCCAAAAGGGTCAGTTGGTTTCTTAGTGTTTGGAGTTG -3' (q3e2R)

SEQ ID NO: 21

5'- GCGGTCCCAAAAGGGTCAGTTGGATCATGGGCCATGTGC -3' (g9e9F)

SEQ ID NO: 22

5'- GCGGTCCCAAAAGGGTCAGTACTACCTTGCCTGCTCCAGTGG -3' (g9e9R)

SEQ ID NO: 23

5'- GCGGTCCCAAAAGGGTCAGTAGGTAGCAGCTATTTTTATGG -3' (g13e2F)

SEQ ID NO: 24

5'- GCGGTCCCAAAAGGGTCAGTTAAGGGAGTCTTTTGCACAA -3' (g13e2R)

SEQ ID NO: 25

5'- GCGGTCCCAAAAGGGTCAGTGCAATTTTGGATGACCTTC -3' (q16i1F)

SEQ ID NO: 26

5'- GCGGTCCCAAAAGGGTCAGTTAGACAGGACTTCAACCCTC -3' (q16i2R)

SEQ ID NO: 27

5'- GCGGTCCCAAAAGGGTCAGTGGTGATTATGGGAGAACTGG -3' (q10e10F)

SEQ ID NO: 28

5'- GCGGTCCCAAAAGGGTCAGTATGCTTTGATGACGCTTC -3' (q10e11R)

SEQ ID NO: 29

5'- GCGGTCCCAAAAGGGTCAGTTTCATTGAAAAGCCCGAC -3' (q19e12F)

SEQ ID NO: 30

5'- GCGGTCCCAAAAGGGTCAGTCACCTTCTGTGTATTTTGCTG -3' (q19e13R)

SEQ ID NO: 31

5'- GCGGTCCCAAAAGGGTCAGTAAGTATTGGACAACTTGTTAGTCTC -3' (q5e12F)

SEQ ID NO: 32

5'- GCGGTCCCAAAAGGGTCAGTCGCCTTTCCAGTTGTATAATTT -3' (q5e13R)

or a complement of one or more of these sequences.

[0020] In another aspect, the present invention provides methods of amplifying CF nucleic acids to determine the presence of one or more mutant CF sequences. In accordance with this method, nucleic acid suspected of containing mutant CF sequences are amplified using one or more primers that flank one or more predetermined nucleic acid sequences that are associated with cystic fibrosis under conditions such that the primers will amplify the predetermined nucleic

acid sequences, if present. In preferred embodiments, the amplification primers used are one or more of the sequences designated as SEQ ID NO: 3 through SEQ ID NO: 32, or a complement of one or more of these sequences. In preferred embodiments, pairs of primers are used for amplification, the pairs being SEQ ID NOs: 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13 and 14, 15 and 16, 17 and 18, 19 and 20, 21 and 22, 23 and 24, 25 and 26, 27 and 28, 29 and 30, and 31 and 32. In further preferred embodiments, the number of pairs of primers is 5 pairs of primers, even more preferably 10 pairs of primers and most preferably 15 pairs of primers.

[0021] In the case where the 15 pairs of primers are used in combinations, primer sets are added in the following ratios determined as the moles (mole is defined as mass/molecular weight of a compound) of primers for exon 12 and 21 (SEQ ID NO: 9, 10, 13 and 14) relative to the moles of each other primer sets, the ratio being about 2 for exons 4 and i19 (SEQ ID NOs; 3-6), about 3.2 for exons 19, 7, 11 and i5 (SEQ ID NOs; 7, 8, 15, 16, and 29-32), about 4 for exons 3 and 14 (SEQ ID NOs; 11, 12, 19, 20), about 4.8 for exons 16, 20, 13 and 10 (SEQ ID NOs; 17, 18, 23 and 28), and about 8 for exon 9 (SEQ ID NOs; 22 and 21). Thus, the amount of exon 12 and 21 primers added is about (SEQ ID NO: 9, 10, 13 and 14) 2 fold that of exons 4 and i19 (SEQ ID NOs; 3-6), about 3.2 fold that of exons 19, 7 and i5 (SEQ ID NOs; 7, 8, 15, 16, and 29-32), about 4 fold that of exons 3 and 14 (SEQ ID NOs; 11, 12, 19, 20), about 4.8 fold that of exons 16, 20, 13 and 10 (SEQ ID NOs; 17, 18, 23 and 28), and about 8 fold that of exon 9 (SEQ ID NOs; 22 and 21).

[0022] The method of identifying the presence or absence of mutant CF sequence by amplification can be used to determine whether a subject has a genotype containing one or more nucleotide sequences correlated with cystic fibrosis. The presence of a wildtype or mutant sequence at each predetermined location can be ascertained by the invention methods.

[0023] By "amplification" is meant one or more methods known in the art for copying a target nucleic acid, thereby increasing the number of copies of a selected nucleic acid sequence. Amplification may be exponential or linear. A target nucleic acid may be either DNA or RNA. The sequences amplified in this manner form an "amplicon." While the exemplary methods described hereinafter relate to amplification using the polymerase chain reaction ("PCR"), numerous other methods are known in the art for amplification of nucleic acids (*e.g.*, isothermal methods, rolling circle methods, *etc.*). The skilled artisan will understand that these other methods may be used either in place of, or together with, PCR methods.

[0024] The nucleic acid suspected of containing mutant CF sequence may be obtained from a biological sample. By "biological sample" is meant a sample obtained from a biological source. A biological sample can, by way of non-limiting example, consist of or comprise blood, sera, urine, feces, epidermal sample, skin sample, cheek swab, sperm, amniotic fluid, cultured cells, bone marrow sample and/or chorionic villi. Convenient biological samples may be obtained by, for example, scraping cells from the surface of the buccal cavity. The term biological sample includes samples which have been processed to release or otherwise make available a nucleic acid for detection as described herein. For example, a biological sample may include a cDNA that has been obtained by reverse transcription of RNA from cells in a biological sample.

[0025] By "subject" is meant a human or any other animal which contains as CFTR gene that can be amplified using the primers and methods described herein. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. A human includes pre and post natal forms. Particularly preferred subjects are humans being tested for the existence of a CF carrier state or disease state.

[0026] By "identifying" with respect to an amplified sample is meant that the presence or absence of a particular nucleic acid amplification product is detected. Numerous methods for detecting the results of a nucleic acid amplification method are known to those of skill in the art.

[0027] In another aspect the present invention provides kits for one of the methods described herein. In various embodiments, the kits contain one or more of the following components in an amount sufficient to perform a method on at least one sample: one or more primers of the present invention, one or more devices for performing the assay, which may include one or more probes that hybridize to a mutant CF nucleic acid sequence, and optionally contain buffers, enzymes, and reagents for performing a method of detecting a genotype of cystic fibrosis in a nucleic acid sample.

[0028] The following description of the background of the invention is provided simply as an aid in understanding the invention and is not admitted to describe or constitute prior art to the invention.

Detailed Description of the Invention

[0029] The present invention provides specific primers that aid in the detection of mutant CF genotype. Such primers enable the amplification of segments of the CFTR gene that are known to contain mutant CF sequence from a nucleic acid containing biological sample. By amplifying specific regions of the CFTR gene, the invention primers facilitate the identification of wildtype or mutant CF sequence at a particular location of the CFTR gene. Accordingly, there is provided a substantially purified nucleic acid sample comprising one or more nucleic acids having sequences selected from the group consisting of:

5'- GCGGTCCCAAAAGGGTCAGTTGTAGGAAGTCACCAAAG -3' (SEQ ID NO: 3), 5'-GCGGTCCCAAAAGGGTCAGTCGATACAGAATATATGTGCC -3' (SEQ ID NO: 4), 5'-GCGGTCCCAAAAGGGTCAGTGAATCATTCAGTGGGTATAAGCAG -3' (SEQ ID NO: 5), 5'- GCGGTCCCAAAAGGGTCAGTCTTCAATGCACCTCCTCCC -3' (SEQ ID NO: 6), 5'-GCGGTCCCAAAAGGGTCAGTTAGATACTTCAATAGCTCAGCC -3' (SEQ ID NO: 7), 5'-GCGGTCCCAAAAGGGTCAGTGGTACATTACCTGTATTTTGTTT -3' (SEQ ID NO: 8), 5'-GCGGTCCCAAAAGGGTCAGTGTGAATCGATGTGGTGACCA -3' (SEQ ID NO: 9), 5'-GCGGTCCCAAAAGGGTCAGTCTGGTTTAGCATGAGGCGGT -3' (SEQ ID NO: 10), 5'-GCGGTCCCAAAAGGGTCAGTTTGGTTGTGCTGTGGCTCCT -3' (SEQ ID NO: 11), 5'-GCGGTCCCAAAAGGGTCAGTACAATACATACAAACATAGTGG -3' (SEQ ID NO: 12), 5'- GCGGTCCCAAAAGGGTCAGTGAAAGTATTTATTTTTCTGGAAC -3' (SEQ ID NO: 13), 5'- GCGGTCCCAAAAGGGTCAGTGTGTGTAGAATGATGTCAGCTAT -3' (SEQ ID NO: 14), 5'- GCGGTCCCAAAAGGGTCAGTCAGATTGAGCATACTAAAAGTG -3' (SEQ ID NO: 15), 5'- GCGGTCCCAAAAGGGTCAGTTACATGAATGACATTTACAGCA -3' (SEQ ID NO: 16), 5'- GCGGTCCCAAAAGGGTCAGTAAGAACTGGATCAGGGAAGA -3' (SEQ ID NO: 17), 5'- GCGGTCCCAAAAGGGTCAGTTCCTTTTGCTCACCTGTGGT -3' (SEQ ID NO: 18), 5'- GCGGTCCCAAAAGGGTCAGTGGTCCCACTTTTTATTCTTTTGC -3' (SEQ ID NO: 19), 5'- GCGGTCCCAAAAGGGTCAGTTGGTTTCTTAGTGTTTGGAGTTG -3' (SEQ ID NO: 20), 5'- GCGGTCCCAAAAGGGTCAGTTGGATCATGGGCCATGTGC -3' (SEQ ID NO: 21), 5'- GCGGTCCCAAAAGGGTCAGTACTACCTTGCCTGCTCCAGTGG -3' (SEQ ID NO: 22), 5'- GCGGTCCCAAAAGGGTCAGTAGGTAGCAGCTATTTTTATGG -3' (SEQ ID NO: 23), 5'- GCGGTCCCAAAAGGGTCAGTTAAGGGAGTCTTTTGCACAA -3' (SEQ ID NO: 24), 5'- GCGGTCCCAAAAGGGTCAGTGCAATTTTGGATGACCTTC -3'

(SEQ ID NO: 25), 5'- GCGGTCCCAAAAGGGTCAGTTAGACAGGACTTCAACCCTC -3' (SEQ ID NO: 26), 5'- GCGGTCCCAAAAGGGTCAGTGGTGATTATGGGAGAACTGG -3' (SEQ ID NO: 27), 5'- GCGGTCCCAAAAGGGTCAGTATGCTTTGATGACGCTTC -3' (SEQ ID NO: 28), 5'- GCGGTCCCAAAAGGGTCAGTTTCATTGAAAAGCCCGAC -3' (SEQ ID NO: 29), 5'- GCGGTCCCAAAAGGGTCAGTCACCTTCTGTGTATTTTGCTG -3' (SEQ ID NO: 30), 5'- GCGGTCCCAAAAGGGTCAGTAAGTATTGGACAACTTGTTAGTCTC -3' (SEQ ID NO: 31), 5'- GCGGTCCCAAAAGGGTCAGTCGCCTTTCCAGTTGTATAATTT -3' (SEQ ID NO: 32), or a complementary nucleic acid sequence thereof.

[0030] The invention nucleic acids are useful for primer-directed amplification of CFTR gene segments known to contain CF mutations. The primers may be used individually or, more preferably in pairs that flank a particular CF gene sequence. Thus, SEQ ID NO: 3, 5'- GCGGTCCCAAAAGGGTCAGTTGTAGGAAGTCACCAAAG -3' (g4e1F), and SEQ ID NO: 4, 5'- GCGGTCCCAAAAGGGTCAGTCGATACAGAATATATGTGCC -3' (g4e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 5, 5'- GCGGTCCCAAAAGGGTCAGTGAATCATTCAGTGGGTATAAGCAG -3' (g19i2F), and SEQ ID NO: 6,5'- GCGGTCCCAAAAGGGTCAGTCTTCAATGCACCTCCTCCC -3' (q19i3R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 7, 5'- GCGGTCCCAAAAGGGTCAGTTAGATACTTCAATAGCTCAGCC -3' (g7e1F), and SEQ ID NO: 8, 5'- GCGGTCCCAAAAGGGTCAGTGGTACATTACCTGTATTTTGTTT -3' (g7e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 9, 5'- GCGGTCCCAAAAGGGTCAGTGTGAATCGATGTGGTGACCA -3' (s12e1F), and SEQ ID NO: 10, 5'- GCGGTCCCAAAAGGGTCAGTCTGGTTTAGCATGAGGCGGT -3' (s12e1R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 11, 5'- GCGGTCCCAAAAGGGTCAGTTTGGTTGTGCTGTGGCTCCT -3' (g14be1F), and SEQ ID NO: 12, 5'- GCGGTCCCAAAAGGGTCAGTACAATACATACAAACATAGTGG -3' (g14be2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 13, 5'- GCGGTCCCAAAAGGGTCAGTGAAAGTATTTATTTTTTCTGGAAC -3' (q21e1F), and SEQ ID NO: 14 5'- GCGGTCCCAAAAGGGTCAGTGTGTGTAGAATGATGTCAGCTAT -3' (q21e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 15, 5'- GCGGTCCCAAAAGGGTCAGTCAGATTGAGCATACTAAAAGTG -3' (g11e1F), and SEQ ID NO: 16, 5'- GCGGTCCCAAAAGGGTCAGTTACATGAATGACATTTACAGCA -3'

(g11e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 17, 5'- GCGGTCCCAAAAGGGTCAGTAAGAACTGGATCAGGGAAGA -3' (g20e1F), and SEQ ID NO: 18, 5'- GCGGTCCCAAAAGGGTCAGTTCCTTTTGCTCACCTGTGGT -3' (g20e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 19, 5'- GCGGTCCCAAAAGGGTCAGTGGTCCCACTTTTTATTCTTTTGC -3' (q3e2F), and SEQ ID NO: 20 5'- GCGGTCCCAAAAGGGTCAGTTGGTTTCTTAGTGTTTGGAGTTG -3' (q3e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 21, 5'- GCGGTCCCAAAAGGGTCAGTTGGATCATGGGCCATGTGC -3' (g9e9F), and SEQ ID NO: 22, 5'- GCGGTCCCAAAAGGGTCAGTACTACCTTGCCTGCTCCAGTGG -3' (g9e9R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 23, 5'- GCGGTCCCAAAAGGGTCAGTAGGTAGCAGCTATTTTTATGG -3' (g13e2F), and SEQ ID NO: 24, 5'- GCGGTCCCAAAAGGGTCAGTTAAGGGAGTCTTTTGCACAA -3' (g13e2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 25 5'- GCGGTCCCAAAAGGGTCAGTGCAATTTTGGATGACCTTC -3' (q16i1F), and SEQ ID NO: 26 5'- GCGGTCCCAAAAGGGTCAGTTAGACAGGACTTCAACCCTC -3' (q16i2R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 27, 5'- GCGGTCCCAAAAGGGTCAGTGGTGATTATGGGAGAACTGG -3' (q10e10F), and SEQ ID NO: 28, 5'- GCGGTCCCAAAAGGGTCAGTATGCTTTGATGACGCTTC -3' (q10e11R), are preferably used together as forward (F) and reverse (R) primers; SEQ ID NO: 29, 5'- GCGGTCCCAAAAGGGTCAGTTTCATTGAAAAGCCCGAC -3' (q19e12F), and SEQ ID NO: 30, 5'- GCGGTCCCAAAAGGGTCAGTCACCTTCTGTGTATTTTGCTG -3' (q19e13R) are preferably used together as forward (F) and reverse (R) primers; and SEQ ID NO: 31, 5'- GCGGTCCCAAAAGGGTCAGTAAGTATTGGACAACTTGTTAGTCTC -3' (q5e12F), and SEQ ID NO: 32, 5'- GCGGTCCCAAAAGGGTCAGTCGCCTTTCCAGTTGTATAATTT -3' (q5e13R), are preferably used together as forward (F) and reverse (R) primers.

[0031] Accordingly, there is provided a method of amplifying a nucleic acid sequence, comprising, contacting a nucleic acid containing sample with reagents suitable for nucleic acid amplification including one or more pairs of primers flanking one or more predetermined nucleic acid sequences that are correlated with cystic fibrosis, and amplifying said one or more predetermined nucleic acid sequences, if present, wherein said primers are one or more pairs of nucleic acids selected from the group consisting of:

5'- GCGGTCCCAAAAGGGTCAGTTGTAGGAAGTCACCAAAG -3' (SEQ ID NO: 3), 5'-GCGGTCCCAAAAGGGTCAGTCGATACAGAATATATGTGCC -3' (SEQ ID NO: 4), 5'-GCGGTCCCAAAAGGGTCAGTGAATCATTCAGTGGGTATAAGCAG -3' (SEQ ID NO: 5), 5'- GCGGTCCCAAAAGGGTCAGTCTTCAATGCACCTCCTCCC -3' (SEQ ID NO: 6), 5'-GCGGTCCCAAAAGGGTCAGTTAGATACTTCAATAGCTCAGCC -3' (SEQ ID NO: 7), 5'-GCGGTCCCAAAAGGGTCAGTGGTACATTACCTGTATTTTGTTT -3' (SEQ ID NO: 8), 5'-GCGGTCCCAAAAGGGTCAGTGTGAATCGATGTGGTGACCA -3' (SEQ ID NO: 9), 5'-GCGGTCCCAAAAGGGTCAGTCTGGTTTAGCATGAGGCGGT -3' (SEQ ID NO: 10), 5'-GCGGTCCCAAAAGGGTCAGTTTGGTTGTGCTGTGGCTCCT -3' (SEQ ID NO: 11), 5'-GCGGTCCCAAAAGGGTCAGTACAATACATACAAACATAGTGG -3' (SEQ ID NO: 12), 5'- GCGGTCCCAAAAGGGTCAGTGAAAGTATTTATTTTTCTGGAAC -3' (SEQ ID NO: 13), 5'- GCGGTCCCAAAAGGGTCAGTGTGTGTAGAATGATGTCAGCTAT -3' (SEQ ID NO: 14), 5'- GCGGTCCCAAAAGGGTCAGTCAGATTGAGCATACTAAAAGTG -3' (SEQ ID NO: 15), 5'- GCGGTCCCAAAAGGGTCAGTTACATGAATGACATTTACAGCA -3' (SEQ ID NO: 16), 5'- GCGGTCCCAAAAGGGTCAGTAAGAACTGGATCAGGGAAGA -3' (SEQ ID NO: 17), 5'- GCGGTCCCAAAAGGGTCAGTTCCTTTTGCTCACCTGTGGT -3' (SEQ ID NO: 18), 5'- GCGGTCCCAAAAGGGTCAGTGGTCCCACTTTTTATTCTTTTGC -3' (SEQ ID NO: 19), 5'- GCGGTCCCAAAAGGGTCAGTTGGTTTCTTAGTGTTTGGAGTTG -3' (SEQ ID NO: 20), 5'- GCGGTCCCAAAAGGGTCAGTTGGATCATGGGCCATGTGC -3' (SEQ ID NO: 21), 5'- GCGGTCCCAAAAGGGTCAGTACTACCTTGCCTGCTCCAGTGG -3' (SEQ ID NO: 22), 5'- GCGGTCCCAAAAGGGTCAGTAGGTAGCAGCTATTTTTATGG -3' (SEQ ID NO: 23), 5'- GCGGTCCCAAAAGGGTCAGTTAAGGGAGTCTTTTGCACAA -3' (SEQ ID NO: 24), 5'- GCGGTCCCAAAAGGGTCAGTGCAATTTTGGATGACCTTC -3' (SEQ ID NO: 25), 5'- GCGGTCCCAAAAGGGTCAGTTAGACAGGACTTCAACCCTC -3' (SEQ ID NO: 26), 5'- GCGGTCCCAAAAGGGTCAGTGGTGATTATGGGAGAACTGG -3' (SEQ ID NO: 27), 5'- GCGGTCCCAAAAGGGTCAGTATGCTTTGATGACGCTTC -3' (SEQ ID NO: 28), 5'- GCGGTCCCAAAAGGGTCAGTTTCATTGAAAAGCCCGAC -3' (SEQ ID NO: 29), 5'- GCGGTCCCAAAAGGGTCAGTCACCTTCTGTGTATTTTGCTG -3' (SEQ ID NO: 30), 5'- GCGGTCCCAAAAGGGTCAGTAAGTATTGGACAACTTGTTAGTCTC -3' (SEQ ID NO: 31), 5'- GCGGTCCCAAAAGGGTCAGTCGCCTTTCCAGTTGTATAATTT -3' (SEQ ID NO: 32). The above pairs of primers have been designed for multiplex use. Thus, one

may simultaneously in a single sample amplify one or more CFTR gene segments. In preferred embodiment, five pairs of primers are used to amplify at least five CFTR gene segments. In a more preferred embodiment, ten pairs may be used and in most preferred embodiment, all 15 pairs of primers may be used.

[0032] The identify of mutations characteristics of each amplified segment for each primer pair are shown in the following table.

[0033] The table below identifies preferred primer pairs and characteristics of the amplified product.

Table 1: CFTR Primer Pairs and Amplicon Characteristics

Forward Primer	Reverse Primer	Exon/Intron	Size
g14be1F (SEQ ID NO. 11)	g14be24 (SEQ ID NO. 12)	14b/i14b	149
q5e12F (SEQ ID NO. 31)	q5e13R (SEQ ID NO. 32)	5/i5	165
g20e1F (SEQ ID NO. 17)	g20e2R (SEQ ID NO. 18)	20	194
q16i1F (SEQ ID NO. 25)	q16i2R (SEQ ID NO. 26)	16/i16	200
q10e10F (SEQ ID NO. 27)	q10e11R (SEQ ID NO. 28)	10	204
q21e1F (SEQ ID NO. 13)	q21e2R (SEQ ID NO. 14)	21	215
g11e1F (SEQ ID NO. 15)	g11e2R (SEQ ID NO. 16)	i10/11/i11	240
g7e1F (SEQ ID NO. 7)	g7e2R (SEQ ID NO. 8)	7	259
g4e1F (SEQ ID NO. 3)	g4e2R (SEQ ID NO. 4)	4/i4	306
q3e2F (SEQ ID NO. 19)	q3e2R (SEQ ID NO. 20)	3/i3	308
q19e12F (SEQ ID NO. 29)	q1913e2R (SEQ ID NO. 30)	i18/19	310
q13e2F (SEQ ID NO. 23)	g13e2R (SEQ ID NO. 24)	13	334
g9e9F (SEQ ID NO. 21)	g9e9R (SEQ ID NO. 22)	i8/9	351
g19i2F (SEQ ID NO. 5)	g19i3R (SEQ ID NO. 6)	i19	389
s12e1F (SEQ ID NO. 9)	s12e1R (SEQ ID NO. 10)	i11/12/i12	465

[0034] The nucleic acid to be amplified may be from a biological sample such as an organism, cell culture, tissue sample, and the like. The biological sample can be from a subject which includes any eukaryotic organism or animal, preferably fungi, invertebrates, insects,

arachnids, fish, amphibians, reptiles, birds, marsupials and mammals. A preferred subject is a human, which may be a patient presenting to a medical provider for diagnosis or treatment of a disease. The biological sample may be obtained from a stage of life such as a fetus, young adult, adult, and the like. Particularly preferred subjects are humans being tested for the existence of a CF carrier state or disease state.

[0035] The sample to be analyzed may consist of or comprise blood, sera, urine, feces, epidermal sample, skin sample, cheek swab, sperm, amniotic fluid, cultured cells, bone marrow sample and/or chorionic villi, and the like. A biological sample may be processed to release or otherwise make available a nucleic acid for detection as described herein. Such processing may include steps of nucleic acid manipulation, e.g., preparing a cDNA by reverse transcription of RNA from the biological sample. Thus, the nucleic acid to be amplified by the methods of the invention may be DNA or RNA.

[0036] Nucleic acid may be amplified by one or more methods known in the art for copying a target nucleic acid, thereby increasing the number of copies of a selected nucleic acid sequence. Amplification may be exponential or linear. The sequences amplified in this manner form an "amplicon." In a preferred embodiment, the amplification by the is by the polymerase chain reaction ("PCR") (e.g., Mullis, K. et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Erlich H. et al., European Patent Appln. 50,424; European Patent Appln. 84,796, European Patent Application 258,017, European Patent Appln. 237,362; Mullis, K., European Patent Appln. 201,184; Mullis K. et al., U.S. Pat. No. 4,683,202; Erlich, H., U.S. Pat. No. 4,582,788; and Saiki, R. et al., U.S. Pat. No. 4,683,194). Other known nucleic acid amplification procedures that can be used include, for example, transcription-based amplification systems or isothermal amplification methods (Malek, L. T. et al., U.S. Pat. No. 5,130,238; Davey, C. et al., European Patent Application 329,822; Schuster et al., U.S. Pat. No. 5,169,766; Miller, H. I. et al., PCT appln. WO 89/06700; Kwoh, D. et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:1173 (1989); Gingeras, T. R. et al., PCT application WO 88/10315; Walker, G. T. et al., Proc. Natl. Acad. Sci. (U.S.A.) 89:392-396 (1992)). Amplification may be performed to with relatively similar levels of each primer of a primer pair to generate an double stranded amplicon. However, asymmetric PCR may be used to amplify predominantly or exclusively a single stranded product as is well known in the art (e.g., Poddar et al. *Molec. And Cell. Probes* 14:25-32 (2000)). This can be achieved for each pair of primers by reducing the concentration of one primer significantly

relative to the other primer of the pair (e.g. 100 fold difference). Amplification by asymmetric PCR is generally linear. One of ordinary skill in the art would know that there are many other useful methods that can be employed to amplify nucleic acid with the invention primers (*e.g.*, isothermal methods, rolling circle methods, *etc.*), and that such methods may be used either in place of, or together with, PCR methods. Persons of ordinary skill in the art also will readily acknowledge that enzymes and reagents necessary for amplifying nucleic acid sequences through the polymerase chain reaction, and techniques and procedures for performing PCR, are well known. The examples below illustrate a standard protocol for performing PCR and the amplification of nucleic acid sequences that correlate with or are indicative of cystic fibrosis.

[0037] In another aspect, the present invention provides methods of detecting a cystic fibrosis genotype in a biological sample. The methods comprise amplifying nucleic acids in a biological sample of the subject and identifying the presence or absence of one or more mutant cystic fibrosis nucleic acid sequences in the amplified nucleic acid. Accordingly, the present invention provides a method of determining the presence or absence of one or more mutant cystic fibrosis nucleic acid sequences in a nucleic acid containing sample, comprising: contacting said sample with reagents suitable for nucleic acid amplification including one or more pairs of nucleic acid primers flanking one or more predetermined nucleic acid sequences that are correlated with cystic fibrosis, amplifying said predetermined nucleic acid sequence(s), if present, to provide an amplified sample; and identifying the presence or absence of said one or more predetermined sequences in said amplified sample, whereby the presence or absence of said one or more mutant cystic fibrosis nucleic acid sequences is determined; wherein said pairs of nucleic acid primers are selected from the group consisting of:

5'- GCGGTCCCAAAAGGGTCAGTTGTAGGAAGTCACCAAAG -3' (SEQ ID NO: 3) and 5'- GCGGTCCCAAAAGGGTCAGTCGATACAGAATATATGTGCC -3' (SEQ ID NO: 4), 5'- GCGGTCCCAAAAGGGTCAGTGAATCATTCAGTGGGTATAAGCAG -3' (SEQ ID NO: 5) and 5'- GCGGTCCCAAAAGGGTCAGTCTTCAATGCACCTCCTCCC -3' (SEQ ID NO: 6), 5'- GCGGTCCCAAAAGGGTCAGTTAGATACTTCAATAGCTCAGCC -3' (SEQ ID NO: 7) and 5'- GCGGTCCCAAAAGGGTCAGTGGTACATTACCTGTATTTTGTTT -3' (SEQ ID NO: 8), 5'- GCGGTCCCAAAAGGGTCAGTGTGAATCGATGTGGTGACCA -3' (SEQ ID NO: 9) and 5'- GCGGTCCCAAAAGGGTCAGTCTGGTTTAGCATGAGGCGGT -3' (SEQ ID NO: 10), 5'- GCGGTCCCAAAAGGGTCAGTTTGGTTGTGCTGTGGCTCCT -3'

(SEQ ID NO: 11) and 5'-
GCGGTCCCAAAAGGGTCAGTACAATACATACAAACATAGTGG -3' (SEQ ID NO: 12), 5'- GCGGTCCCAAAAGGGTCAGTGAAAGTATTTATTTTTTCTGGAAC -3' (SEQ ID NO: 13) and 5'- GCGGTCCCAAAAGGGTCAGTGTGTGTAGAATGATGTCAGCTAT -3' (SEQ ID NO: 14), 5'- GCGGTCCCAAAAGGGTCAGTCAGATTGAGCATACTAAAAGTG -3' (SEQ ID NO: 15) and 5'-
GCGGTCCCAAAAGGGTCAGTTACATGAATGACATTTACAGCA -3' (SEQ ID NO: 16), 5'- GCGGTCCCAAAAGGGTCAGTAAGAACTGGATCAGGGAAGA -3' (SEQ ID NO: 17) and 5'- GCGGTCCCAAAAGGGTCAGTTCCTTTTGCTCACCTGTGGT -3' (SEQ ID NO: 18), 5'- GCGGTCCCAAAAGGGTCAGTGGTCCCACTTTTTATTCTTTTGC -3' (SEQ ID NO: 19) and 5'- GCGGTCCCAAAAGGGTCAGTTGGTTTCTTAGTGTTTGGAGTTG -3' (SEQ ID NO: 20), 5'- GCGGTCCCAAAAGGGTCAGTTGGATCATGGGCCATGTGC -3' (SEQ ID NO: 21) and 5'- GCGGTCCCAAAAGGGTCAGTACTACCTTGCCTGCTCCAGTGG -3' (SEQ ID NO: 22), 5'- GCGGTCCCAAAAGGGTCAGTAGGTAGCAGCTATTTTTATGG -3' (SEQ ID NO: 23) and 5'- GCGGTCCCAAAAGGGTCAGTTAAGGGAGTCTTTTGCACAA -3' (SEQ ID NO: 24), 5'- GCGGTCCCAAAAGGGTCAGTGCAATTTTGGATGACCTTC -3' (SEQ ID NO: 25) and 5'- GCGGTCCCAAAAGGGTCAGTTAGACAGGACTTCAACCCTC -3' (SEQ ID NO: 26), 5'- GCGGTCCCAAAAGGGTCAGTGGTGATTATGGGAGAACTGG -3' (SEQ ID NO: 27) and 5'- GCGGTCCCAAAAGGGTCAGTATGCTTTGATGACGCTTC -3' (SEQ ID NO: 28), 5'- GCGGTCCCAAAAGGGTCAGTTTCATTGAAAAGCCCGAC -3' (SEQ ID NO: 29) and 5'- GCGGTCCCAAAAGGGTCAGTCACCTTCTGTGTATTTTGCTG -3' (SEQ ID NO: 30), and 5'-
GCGGTCCCAAAAGGGTCAGTAAGTATTGGACAACTTGTTAGTCTC -3' (SEQ ID NO: 31) and 5'- GCGGTCCCAAAAGGGTCAGTCGCCTTTCCAGTTGTATAATTT -3' (SEQ ID NO: 32).

[0038] One may analyze the amplified product for the presence of absence of any of a number of mutant CF sequences that may be present in the sample nucleic acid. As already discussed, numerous mutations in the CFTR gene have been associated with CF carrier and disease states. For example, a three base pair deletion leading to the omission of a phenylalanine residue in the gene product has been determined to correspond to the mutations of the CF gene in approximately 70% of the patients affected by CF. The table below identifies preferred CF

sequences and identifies which of the primer pairs of the invention may be used to amplify the sequence.

Table 2: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO:19 and 20.

Name	Nucleotide_change	Exon	Consequence
297-3C->T	C to T at 297-3	intron 2	mRNA splicing defect?
E56K	G to A at 298	3	Glu to Lys at 56
300delA	deletion of A at 300	3	Frameshift
W57R	T to C at 301	3	Trp to Arg at 57
W57G	T to G at 301	3	Trp to Gly at 57
W57X(TAG)	G to A at 302	3	Trp to Stop at 57
W57X(TGA)	G to A at 303	3	Trp to Stop at 57
D58N	G to A at 304	3	Asp to Asn at 58
D58G	A to G at 305	3	Asp to Gly at 58
306insA	insertion of A at 306	3	Frameshift
306delTAGA	deletion of TAGA from 306	3	Frameshift
E60L	G to A at 310	3	Glu to Leu at 60
E60X	G to T at 310	3	Glu to Stop at 60
E60K	G to A at 310	3	Glu to Lys at 60
N66S	A to G at 328	3	Asn to Ser at 66
P67L	C to T at 332	3	Pro to Leu at 67
K68E	A to G at 334	3	Lys to Glu at 68
K68N	A to T at 336	3	Lys to Asn at 68
A72T	G to A at 346	3	Ala to Thr at 72
A72D	C to A at 347	3	Ala to Asp at 72
347delC	deletion of C at 347	3	Frameshift
R74W	C to T at 352	3	Arg to Trp at 74
R74Q	G to A at 353	3	Arg to Gln at 74
R75X	C to T at 355	3	Arg to Stop at 75
R75L	G to T at 356	3	Arg to Leu at 75
359insT	insertion of T after 359	3	Frameshift
360delT	deletion of T at 360	3	Frameshift
W79R	T to C at 367	3	Trp to Arg at 79
W79X	G to A at 368	3	Trp to Stop at 79
G85E	G to A at 386	3	Gly to Glu at 85

G85V	G to T at 386	3	Gly to Val at 85
F87L	T to C at 391	3	Phe to Leu at 87
394delTT	deletion of TT from 394	3	frameshift
L88S	T to C at 395	3	Leu to Ser at 88
L88X(T->A)	T to A at 395	3	Leu to Stop at 88
L88X(T->G)	T to G at 395	3	Leu to Stop at 88
Y89C	A to G at 398	3	Tyr to Cys at 89
L90S	T to C at 401	3	Leu to Ser at 90
G91R	G to A at 403	3	Gly to Arg at 91
405+1G->A	G to A at 405+1	intron 3	mRNA splicing defect
405+3A->C	A to C at 405+3	intron 3	mRNA splicing defect?
405+4A->G	A to G at 405+4	intron 3	mRNA splicing defect?

Table 3: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 3 and 4.

Name	Nucleotide_change	Exon	Consequence
A96E	C to A at 419	4	Ala to Glu at 96
Q98X	C to T at 424	4	Gln to Stop at 98 (Pakistani specific?)
Q98P	A to C at 425	4	Gln to Pro at 98
Q98R	A to G at 425	4	Gln to Arg at 98
P99L	C to T at 428	4	Pro to Leu at 99
L101X	T to G at 434	4	Leu to Stop at 101
435insA	insertion of A after 435	4	Frameshift
G103X	G to T at 439	4	Gly to Stop at 103
441delA	deletion of A at 441 and T to A at 486	4	Frameshift
444delA	deletion of A at 444	4	Frameshift
I105N	T to A at 446	4	Ile to Asn at 105
451del8	deletion of GCTTCCTA from 451	4	Frameshift
S108F	C to T at 455	4	Ser to Phe at 108
457TAT->G	TAT to G at 457	4	Frameshift
Y109N	T to A at 457	4	Tyr to Asn at 109
458delAT	deletion of AT at 458	4	Frameshift
Y109C	A to G at 458	4	Tyr to Cys at 109
460delG	deletion of G at 460	4	Frameshift
D110Y	G to T at 460	4	Asp to Tyr at 110

D110H	G to C at 460	4	Asp to His at 110
D110E	C to A at 462	4	Asp to Glu at 110
P111A	C to G at 463	4	Pro to Ala at 111
P111L	C to T at 464	4	Pro to Leu at 111
[delta]E115	3 bp deletion of 475-477	4	deletion of Glu at 115
E116Q	G to C at 478	4	Glu to Gln at 116
E116K	G to A at 478	4	Glu to Lys at 116
R117C	C to T at 481	4	Arg to Cys at 117
R117P	G to C at 482	4	Arg to Pro at 117
R117L	G to T at 482	4	Arg to Leu at 117
R117H	G to A at 482	4	Arg to His at 117
I119V	A to G at 487	4	Iso to Val at 119
A120T	G to A at 490	4	Ala to Thr at 120
Y122X	T to A at 498	4	Tyr to Stop at 122
I125T	T to C at 506	4	Ile to Thr at 125
G126D	G to A at 509	4	Gly to Asp at 126
L127X	T to G at 512	4	Leu to Stop at 127
525delT	deletion of T at 525	4	Frameshift
541del4	deletion of CTCC from 541	4	Frameshift
541delC	deletion of C at 541	4	Frameshift
L137R	T to G at 542	4	Leu to Arg at 137
L137H	T to A at 542	4	Leu to His at 137
L138ins	insertion of CTA, TAC or ACT at nucleotide 544, 545 or 546	4	insertion of leucine at 138
546insCTA	insertion of CTA at 546	4	Frameshift
547insTA	insertion of TA after 547	4	Frameshift
H139L	A to T at 548	4	His to Leu at 548
H139R	A to G at 548	4	His to Arg at 139
P140S	C to T at 550	4	Pro to Ser at 140
P140L	C to T at 551	4	Pro to Leu at 140
552insA	insertion of A after 552	4	Frameshift
A141D	C to A at 554	4	Ala to Asp at 141
556delA	deletion of A at 556	4	Frameshift
557delT	deletion of T at 557	4	Frameshift
565delC	deletion of C at 565	4	Frameshift
H146R	A to G at 569	4	His to Arg at 146 (CBAVD)
574delA	deletion of A at 574	4	Frameshift

I148N	T to A at 575	4	Ile to Asn at 148
I148T	T to C at 575	4	Ile to Thr at 148
G149R	G to A at 577	4	Gly to Arg at 149
Q151X	C to T at 583	4	Gln to Stop at 151
M152V	A to G at 586	4	Met to Val at 152 (mutation?)
M152R	T to G at 587	4	Met to Arg at 152
591del18	deletion of 18 bp from 591	4	deletion of 6 a.a. from
A155P	G to C at 595	4	Ala to Pro at 155
S158R	A to C at 604	4	Ser to Arg at 158
605insT	insertion of T after 605	4	Frameshift
L159X	T to A at 608	4	Leu to Stop at 159
Y161D	T to G at 613	4	Tyr to Asp at 161
Y161N	T to A at 613	4	Tyr to Asn at 161
Y161S	A to C at 614 (together with 612T/A)	4	Tyr to Ser at 161
K162E	A to G at 616	4	Lys to Glu at 162
621G->A	G to A at 621	4	mRNA splicing defect
621+1G->T	G to T at 621+1	intron 4	mRNA splicing defect
621+2T->C	T to C at 621+2	intron 4	mRNA splicing defect
621+2T->G	T to G at 621+2	intron 4	mRNA splicing defect
621+3A->G	A to G at 621+3	intron 4	mRNA splicing defect

Table 4: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 31 and 32.

Name	Nucleotide_change	Exon	Consequence
681delC	deletion of C at 681	5	Frameshift
N186K	C to A at 690	5	Asn to Lys at 186
N187K	C to A at 693	5	Asn to Lys at 187
[delta]D192	deletion of TGA or GAT from 706 or 707	5	deletion of Asp at 192
D192N	G to A at 706	5	Asp to Asn at 192
D192G	A to G at 707	5	Asp to Gly at 192
E193K	G to A at 709	5	Glu to Lys at 193
E193X	G to T at 709	5	Glu to Stop at 193
711+1G->T	G to T at 711+1	intron 5	mRNA splicing defect
711+3A->G	A to G at 711+3	intron 5	mRNA splicing defect
711+3A->C	A to C at 711+3	intron 5	mRNA splicing defect

711+3A->T	A to T at 711+3	intron 5	mRNA splicing defect?
711+5G->A	G to A at 711+5	intron 5	mRNA splicing defect
711+34A->G	A to G at 711+34	intron 5	mRNA splicing defect?

Table 5: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 7 and 8.

Name	Nucleotide_change	Exon	Consequence
[delta]F311	deletion of 3 bp between 1059 and 1069	7	deletion of Phe310, 311 or 312
F311L	C to G at 1065	7	Phe to Leu at 311
G314R	G to C at 1072	7	Gly to Arg at 314
G314E	G to A at 1073	7	Gly to Glu at 314
G314V	G to T at 1073	7	Gly to Val at 324
F316L	T to G at 1077	7	Phe to Leu at 316
1078delT	deletion of T at 1078	7	Frameshift
V317A	T to C at 1082	7	Val to Ala at 317
L320V	T to G at 1090	7	Leu to Val at 320 CAVD
L320X	T to A at 1091	7	Leu to Stop at 320
L320F	A to T at 1092	7	Leu to Phe at 320
V322A	T to C at 1097	7	Val to Ala at 322 (mutation?)
1112delT	deletion of T at 1112	7	Frameshift
L327R	T to G at 1112	7	Leu to Arg at 327
1119delA	deletion of A at 1119	7	Frameshift
G330X	G to T at 1120	7	Gly to Stop at 330
R334W	C to T at 1132	7	Arg to Trp at 334
R334Q	G to A at 1133	7	Arg to Gln at 334
R334L	G to T at 1133	7	Arg to Leu at 334
1138insG	insertion of G after 1138	7	Frameshift
I336K	T to A at 1139	7	Ile to Lys at 336
T338I	C to T at 1145	7	Thr to Ile at 338
1150delA	deletion of A at 1150	7	Frameshift
1154insTC	insertion of TC after 1154	7	Frameshift
1161insG	insertion of G after 1161	7	Frameshift
1161delC	deletion of C at 1161	7	Frameshift
L346P	T to C at 1169	7	Leu to Pro at 346
R347C	C to T at 1171	7	Arg to Cys at 347
R347H	G to A at 1172	7	Arg to His at 347
R347L	G to T at 1172	7	Arg to Leu at 347

R347P	G to C at 1172	7	Arg to Pro at 347
M348K	T to A at 1175	7	Met to Lys at 348
A349V	C to T at 1178	7	Ala to Val at 349
R352W	C to T at 1186	7	Arg to Trp at 352
R352Q	G to A at 1187	7	Arg to Gln at 352
Q353X	C to T at 1189	7	Gln to Stp at 353
Q353H	A to C at 1191	7	Gln to His at 353
1199delG	deletion of G at 1199	7	Frameshift
W356X	G to A at 1200	7	Trp to Stop at 356
Q359K/T360K	C to A at 1207 and C to A at 1211	7	Glu to Lys at 359 and Thr to Lys at 360
Q359R	A to G at 1208	7	Gln to Arg at 359
1213delT	deletion of T at 1213	7	Frameshift
W361R(T->C)	T to C at 1213	7	Trp to Arg at 361
W361R(T->A)	T to A at 1213	7	Trp to Arg at 361
1215delG	deletion of G at 1215	7	Frameshift
1221delCT	deletion of CT from 1221	7	Frameshift
S364P	T to C at 1222	7	Ser to Pro at 364
L365P	T to C at 1226	7	Leu to Pro at 365

Table 6: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 21 and 22.

Name	Nucleotide_change	Exon	Consequence
1342-11TTT->G	TTT to G at 1342-11	intron 8	mRNA splicing defect?
1342-2delAG	deletion of AG from 1342-2	intron 8	Frameshift
1342-2A->C	A to C at 1342-2	intron 8	mRNA splicing defect
1342-1G->C	G to C at 1342-1	intron 8	mRNA splicing defect
E407V	A to T at 1352	9	Glu to Val at 407
1366delG	deletion of G at 1366	9	Frameshift
1367delC	deletion of C at 1367	9	Frameshift
1367del5	deletion of CAAAA at 1367	9	Frameshift
Q414X	C to T at 1372	9	Gln to Stop at 414
N418S	A to G at 1385	9	Asn to Ser at 418
G424S	G to A at 1402	9	Gly to Ser at 424
S434X	C to G at 1433	9	Ser to Stop at 434
D443Y	G to T at 1459	9	Asp to Tyr at 443

1460delAT	deletion of AT from 1460	9	Frameshift
1461ins4	insertion of AGAT after 1461	9	Frameshift
I444S	T to G at 1463	9	Ile to Ser at 444
1471delA	deletion of A at 1471	9	Frameshift
Q452P	A to C at 1487	9	Gln to Pro at 452
[delta]L453	deletion of 3 bp between 1488 and 1494	9	deletion of Leu at 452 or 454
A455E	C to A at 1496	9	Ala to Glu at 455
V456F	G to T at 1498	9	Val to Phe at 456

Table 7: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 27 and 28.

Name	Nucleotide_change	Exon	Consequence
G480C	G to T at 1570	10	Gly to Cys at 480
G480D	G to A at 1570	10	Gly to Asp at 480
G480S	G to A at 1570	10	Gly to Ser at 480
1571delG	deletion of G at 1571	10	Frameshift
1576insT	insertion of T at 1576	10	Framshift
H484Y	C to T at 1582	10	His to Tyr at 484 (CBAVD?)
H484R	A to G at 1583	10	His to Arg at 484
S485C	A to T at 1585	10	Ser to Cys at 485
G486X	G to T at 1588	10	Glu to Stop at 486
S489X	C to A at 1598	10	Ser to Stop at 489
1601delTC	deletion of TC from 1601 or CT from 1602	10	Frameshift
C491R	T to C at 1603	10	Cys to Arg at 491
S492F	C to T at 1607	10	Ser to Phe at 492
Q493X	C to T at 1609	10	Gln to Stop at 493
1609delCA	deletion of CA from 1609	10	Frameshift
Q493R	A to G at 1610	10	Gln to Arg at 493
1612delTT	deletion of TT from 1612	10	Frameshift
W496X	G to A at 1619	10	Trp to Stop at 496
P499A	C to G at 1627	10	Pro to Ala at 499 (CBAVD)
T501A	A to G at 1633	10	Thr to Ala at 501
I502T	T to C at 1637	10	Ile to Thr at 502
I502N	T to A at 1637	10	Ile to Asn at 502

E504X	G to T at 1642	10	Glu to Stop at 504
E504Q	G to C at 1642	10	Glu to Gln at 504
I506L	A to C at 1648	10	Ile to Leu at 506
[delta]I507	deletion of 3 bp between 1648 and 1653	10	deletion of Ile506 or Ile507
I506S	T to G at 1649	10	Ile to Ser at 506
I506T	T to C at 1649	10	Ile to Thr at 506
[delta]F508	deletion of 3 bp between 1652 and 1655	10	deletion of Phe at 508
F508S	T to C at 1655	10	Phe to Ser at 508
D513G	A to G at 1670	10	Asp to Gly at 513 (CBAVD)
1677delTA	deletion of TA from 1677	10	frameshift
Y517C	A to G at 1682	10	Tyr to Cys at 517

Table 8: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 15 and 16.

Name	Nucleotide_change	Exon	Consequence
1716-1G->A	G to A at 1716-1	intron 10	mRNA splicing defect
1717-8G->A	G to A at 1717-8	intron 10	mRNA splicing defect?
1717-3T->G	T to G at 1717-3	intron 10	mRNA splicing defect?
1717-2A->G	A to G at 1717-2	intron 10	mRNA splicing defect
1717-1G->A	G to A at 1717-1	intron 10	mRNA splicing defect
D529H	G to C at 1717	11	Asp to His at 529
1717-9T->A	T to A at 1717-9	intron 10	mRNA splicing mutation?
A534E	C to A at 1733	11	Ala to Glu at 534
1742delAC	deletion of AC from 1742	11	Frameshift
I539T	T to C at 1748	11	Ile to Thr at 539
1749insTA	insertion of TA at 1749	11	frameshift resulting in premature termination at 540
G542X	G to T at 1756	11	Gly to Stop at 542
G544S	G to A at 1762	11	Gly to Ser at 544
G544V	G to T at 1763	11	Gly to Val at 544 (CBAVD)
1774delCT	deletion of CT from 1774	11	Frameshift
S549R(A->C)	A to C at 1777	11	Ser to Arg at 549
S549I	G to T at 1778	11	Ser to Ile at 549
S549N	G to A at 1778	11	Ser to Asn at 549
S549R(T->G)	T to G at 1779	11	Ser to Arg at 549

G550X	G to T at 1780	11	Gly to Stop at 550
G550R	G to A at 1780	11	Gly to Arg at 550
1782delA	deletion of A at 1782	11	Frameshift
G551S	G to A at 1783	11	Gly to Ser at 551
1784delG	deletion of G at 1784	11	Frameshift
G551D	G to A at 1784	11	Gly to Asp at 551
Q552X	C to T at 1786	11	Gln to Stop at 552
Q552K	C to A at 1786	11	Gln to Lys
1787delA	deletion of A at position 1787 or 1788	11	frameshift, stop codon at 558
R553G	C to G at 1789	11	Arg to Gly at 553
R553X	C to T at 1789	11	Arg to Stop at 553
R553Q	G to A at 1790	11	Arg to Gln at 553 (associated with [delta]F508;
R555G	A to G at 1795	11	Arg to Gly at 555
I556V	A to G at 1798	11	Ile to Val at 556 (mutation?)
1802delC	deletion of C at 1802	11	Frameshift
L558S	T to C at 1805	11	Leu to Ser at 558
1806delA	deletion of A at 1806	11	Frameshift
A559T	G to A at 1807	11	Ala to Thr at 559
A559E	C to A at 1808	11	Ala to Glu at 559
R560T	G to C at 1811	11	Arg to Thr at 560; mRNA splicing defect?
R560K	G to A at 1811	11	Arg to Lys at 560
1811+1G->C	G to C at 1811+1	intron 11	mRNA splicing defect
1811+1.6kbA->G	A to G at 1811+1.2kb	intron 11	creation of splice donor site
1811+18G->A	G to A at 1811+18	intron 11	mRNA splicing defect?

Table 9: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 9 and 10.

Name	Nucleotide_change	Exon	Consequence
1812-1G->A	G to A at 1812-1	intron 11	mRNA splicing defect
R560S	A to C at 1812	12	Arg to Ser at 560
1813insC	insertion of C after 1813 (or 1814)	12	Frameshift
A561E	C to A at 1814	12	Ala to Glu at 561
V562I	G to A at 1816	12	Val to Ile at 562
V562L	G to C at 1816	12	Val to Leu at 562
Y563D	T to G at 1819	12	Tyr to Asp at 563

Y563N	T to A at 1819	12	Tyr to Asn at 563
Y563C	A to G at 1821	12	Tyr to Cys at 563
1833delT	deletion of T at 1833	12	Frameshift
L568X	T to A at 1835	12	Leu to Stop at 568
L568F	G to T at 1836	12	Leu to Phe at 568 (CBAVD?)
Y569D	T to G at 1837	12	Tyr to Asp at 569
Y569H	T to C at 1837	12	Tyr to His at 569
Y569C	A to G at 1838	12	Tyr to Cys at 569
V569X	T to A at 1839	12	Tyr to Stop at 569
L571S	T to C at 1844	12	Leu to Ser at 571
1845delAG/1846delGA	deletion of AG at 1845 or GA at 1846	12	Frameshift
D572N	G to A at 1846	12	Asp to Asn at 572
P574H	C to A at 1853	12	Pro to His at 574
G576X	G to T at 1858	12	Gly to Stop at 576
G576A	G to C at 1859	12	Gly to Ala at 576 (CAVD)
Y577F	A to T at 1862	12	Tyr to Phe at 577
D579Y	G to T at 1867	12	Asp to Tyr at 579
D579G	A to G at 1868	12	Asp to Gly at 579
D579A	A to C at 1868	12	Asp to Ala at 579
1870delG	deletion of G at 1870	12	Frameshift
1874insT	insertion of T between 1871 and 1874	12	Frameshift
T582R	C to G at 1877	12	Thr to Arg at 582
T582I	C to T at 1877	12	Thr to Ile at 582
E585X	G to T at 1885	12	Glu to Stop at 585
S589N	G to A at 1898	12	Ser to Asn at 589 (mRNA splicing defect?)
S589I	G to T at 1898	12	Ser to Ile at 589 (splicing?)
1898+1G->A	G to A at 1898+1	intron 12	mRNA splicing defect
1898+1G->C	G to C at 1898+1	intron 12	mRNA splicing defect
1898+1G->T	G to T at 1898+1	intron 12	mRNA splicing defect
1898+3A->G	A to G at 1898+3	intron 12	mRNA splicing defect?
1898+3A->C	A to C at 1898+3	intron 12	mRNA splicing defect?
1898+5G->A	G to A at 1898+5	intron 12	mRNA splicing defect
1898+5G->T	G to T at 1898+5	intron 12	mRNA splicing defect
1898+73T->G	T to G at 1898+73	intron 12	mRNA splicing defect?

Table 10: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 23 and 24.

Name	Nucleotide_change	Exon	Consequence
1918delGC	deletion of GC from 1918	13	Frameshift
1924del7	deletion of 7 bp (AAACTA) from 1924	13	Frameshift
R600G	A to G at 1930	13	Arg to Gly at 600
I601F	A to T at 1933	13	Ile to Phe at 601
V603F	G to T at 1939	13	Val to Phe at 603
T604I	C to T at 1943	13	Thr to Ile at 604
1949del84	deletion of 84 bp from 1949	13	deletion of 28 a.a. (Met607 to Gln634)
H609R	A to G at 1958	13	His to Arg at 609
L610S	T to C at 1961	13	Leu to Ser at 610
A613T	G to A at 1969	13	Ala to Thr at 613
D614Y	G to T at 1972	13	Asp to Tyr 614
D614G	A to G at 1973	13	Asp to Gly at 614
I618T	T to C at 1985	13	Ile to Thr at 618
L619S	T to C at 1988	13	Leu to Ser at 619
H620P	A to C at 1991	13	His to Pro at 620
H620Q	T to G at 1992	13	His to Gln at 620
G622D	G to A at 1997	13	Gly to Asp at 622 (oligospermia)
G628R(G->A)	G to A at 2014	13	Gly to Arg at 628
G628R(G->C)	G to C at 2014	13	Gly to Arg at 628
L633P	T to C at 2030	13	Leu to Pro at 633
Q634X	T to A at 2032	13	Gln to Stop at 634
L636P	T to C at 2039	13	Leu to Pro at 636
Q637X	C to T at 2041	13	Gln to Stop at 637
2043delG	deletion of G at 2043	13	Frameshift
2051delTT	deletion of TT from 2051	13	Frameshift
2055del9->A	deletion of 9 bp CTCAAAACT to A at 2055	13	Frameshift
D648V	A to T at 2075	13	Asp to Val at 648
D651N	G to A at 2083	13	Asp to Asn at 651
E656X	T to G at 2098	13	Glu to Stop at 656
2108delA	deletion of A at 2108	13	Frameshift

2109del9->A	deletion of 9bp from 2109 and insertion of A	13	Frameshift
2113delA	deletion of A at 2113	13	Frameshift
2116delCTAA	deletion of CTAA at 2116	13	Frameshift
2118del4	deletion of AACT from 2118	13	Frameshift
E664X	G to T at 2122	13	Glu to Stop at 664
T665S	A to T at 2125	13	Thr to Ser at 665
2141insA	insertion of A after 2141	13	Frameshift
2143delT	deletion of T at 2143	13	Frameshift
E672del	deletion of 3 bp between 2145-2148	13	deletion of Glu at 672
G673X	G to T at 2149	13	Gly to Stop at 673
W679X	G to A at 2168	13	Trp to stop at 679
2176insC	insertion of C after 2176	13	Frameshift
K683R	A to G at 2180	13	Lys to Arg at 683
2183AA->G	A to G at 2183 and deletion of A at 2184	13	Frameshift
2183delAA	deletion of AA at 2183	13	Frameshift
2184delA	deletion of A at 2184	13	frameshift
2184insG	inserion of G after 2184	13	Frameshift
2184insA	insertion of A after 2184	13	Frameshift
2185insC	insertion of C at 2185	13	Frameshift
Q685X	C to T at 2185	13	Gln to Stop at 685
E692X	G to T at 2206	13	Glu to Stop at 692
F693L(CTT)	T to C at 2209	13	Phe to Leu at 693
F693L(TTG)	T to G at 2211	13	Phe to Leu at 693
2215insG	insertion of G at 2215	13	Frameshift
K698R	A to G 2225	13	Lys to Arg at 698
R709X	C to T at 2257	13	Arg to Stop at 709
K710X	A to T at 2260	13	Lys to Stop at 710
K716X	AA to GT at 2277 and 2278	13	Lys to Stop at 716
L719X	T to A at 2288	13	Leu to Stop at 719
Q720X	C to T at 2290	13	Gln to stop codon at 720
E725K	G to A at 2305	13	Glu to Lys at 725
2307insA	insertion of A after 2307	13	Frameshift
E730X	G to T at 2320	13	Glu to Stop at 730
L732X	T to G at 2327	13	Leu to Stop at 732
2335delA	deletion of A at 2335	13	Frameshift

R735K	G to A at 2336	13	Arg to Lys at 735
2347delG	deletion of G at 2347	13	Frameshift
2372del8	deletion of 8 bp from 2372	13	Frameshift
P750L	C to T at 2381	13	Pro to Leu at 750
V754M	G to A at 2392	13	Val to Met at 754
T760M	C to T at 2411	13	Thr to Met at 760
R764X	C to T at 2422	13	Arg to Stop at 764
2423delG	deletion of G at 2423	13	Frameshift
R766M	G to T at 2429	13	Arg to Met at 766
2456delAC	deletion of AC at 2456	13	Frameshift
S776X	C to G at 2459	13	Ser to Stop at 776

Table 11: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 11 and 12.

Name	Nucleotide_change	Exon	Consequence
T908N	C to A at 2788	14b	Thr to Asn at 908
2789+2insA	insertion of A after 2789+2	intron 14b	mRNA splicing defect? (CAVD)
2789+3delG	deletion of G at 2789+3	intron 14b	mRNA splicing defect
2789+5G->A	G to A at 2789+5	intron 14b	mRNA splicing defect

Table 12: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 25 and 26.

Name	Nucleotide_change	Exon	Consequence
3100insA	insertion of A after 3100	16	Frameshift
I991V	A to G at 3103	16	Ile to Val at 991
D993Y	G to T at 3109	16	Asp to Tyr at 993
F994C	T to G at 3113	16	Phe to Cys at 994
3120G->A	G to A at 3120	16	mRNA splicing defect
3120+1G->A	G to A at 3120+1	intron 16	mRNA splicing defect

Table 13: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 29 and 30.

Name	Nucleotide_change	Exon	Consequence
3601-20T->C	T to C at 3601-20	intron 18	mRNA splicing mutant?
3601-17T->C	T to C at 3601-17	intron 18	mRNA splicing defect?
3601-2A->G	A to G at 3601-2	intron 18	mRNA splicing defect
R1158X	C to T at 3604	19	Arg to Stop at 1158
S1159P	T to C at 3607	19	Ser to Pro at 115p
S1159F	C to T at 3608	19	Ser to Phe at 1159
R1162X	C to T at 3616	19	Arg to Stop at 1162
3622insT	insertion of T after 3622	19	Frameshift
D1168G	A to G at 3635	19	Asp to Gly at 1168
3659delC	deletion of C at 3659	19	Frameshift
K1177X	A to T at 3661	19	Lys to Stp at 3661 (premature termination)
K1177R	A to G at 3662	19	Lys to Arg at 1177
3662delA	deletion of A at 3662	19	Frameshift
3667del4	deletion of 4 bp from 3667	19	Frameshift
3667ins4	insertion of TCAA after 3667	19	Frameshift
3670delA	deletion of A at 3670	19	Frameshift
Y1182X	C to G at 3678	19	Tyr to Stop at 1182
Q1186X	C to T at 3688	19	Gln to Stop codon at 1186
3696G/A	G to A at 3696	18	No change to Ser at 1188
V1190P	T to A at 3701	19	Val to Pro at 1190
S1196T	C or Q at 3719	19	Ser-Top at 1196
S1196X	C to G at 3719	19	Ser to Stop at 1196
3724delG	deletion of G at 3724	19	Frameshift
3732delA	deletion of A at 3732 and A to G at 3730	19	frameshift and Lys to Glu at 1200
3737delA	deletion of A at 3737	19	Frameshift
W1204X	G to A at 3743	19	Trp to Stop at 1204
S1206X	C to G at 3749	19	Ser to Stop at 1206
3750delAG	deletion of AG from 3750	19	Frameshift
3755delG	deletion of G between 3751 and 3755	19	Frameshift
M1210I	G to A at 3762	19	Met to Ile at 1210
V1212I	G to A at 3766	19	Val to Ile at 1212

Table 14: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 5 and 6.

Name	Nucleotide_change	Exon	Consequence
3849+10kbC->T	C to T in a 6.2 kb EcoRI fragment 10 kb from 19	intron 19	creation of splice acceptor site

Table 15: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 17 and 18.

Name	Nucleotide_change	Exon	Consequence
T1252P	A to C at 3886	20	Thr to Pro at 1252
L1254X	T to G at 3893	20	Leu to Stop at 1254
S1255P	T to C at 3895	20	Ser to Pro at 1255
S1255L	C to T at 3896	20	Ser to Leu at 1255
S1255X	C to A at 3896 and A to G at 3739 in exon 19	20	Ser to Stop at 1255 and Ile to Val at 1203
3898insC	insertion of C after 3898	20	Frameshift
F1257L	T to G at 3903	20	Phe to Leu at 1257
3905insT	insertion of T after 3905	20	Frameshift
3906insG	insertion of G after 3906	20	Frameshift
[delta]L1260	deletion of ACT from either 3909 or 3912	20	deletion of Leu at 1260 or 1261
3922del10->C	deletion of 10 bp from 3922 and replacement with 3921	20	deletion of Glu1264 to Glu1266
I1269N	T to A at 3938	20	Ile to Asn at 1269
D1270N	G to A at 3940	20	Asp to Asn at 1270
3944delGT	deletion of GT from 3944	20	Frameshift
W1274X	G to A at 3954	20	Trp to Stop at 1274
Q1281X	C to T at 3973	20	Gln to Stop at 1281
W1282R	T to C at 3976	20	Trp to Arg at 1282
W1282G	T to G at 3976	20	Trp to Gly at 1282
W1282X	G to A at 3978	20	Trp to Stop at 1282
W1282C	G to T at 3978	20	Trp to Cys at 1282
R1283M	G to T at 3980	20	Arg to Met at 1283
R1283K	G to A at 3980	20	Arg to Lys at 1283
F1286S	T to C at 3989	20	Phe to Ser at 1286

Table 16: CFTR mutations that may be detected in amplified product using as the primer pair SEQ ID NO: 13 and 14.

Name	Nucleotide_change	Exon	Consequence
T1299I	C to T at 4028	21	Thr to Ile at 1299
F1300L	T to C at 4030	21	Phe to Leu at 1300
N1303H	A to C at 4039	21	Asn to His at 1303
N1303I	A to T at 4040	21	Asn to Ile at 1303
4040delA	deletion of A at 4040	21	Frameshift
N1303K	C to G at 4041	21	Asn to Lys at 1303
D1305E	T to A at 4047	21	Asp to Glu at 1305
4048insCC	insertion of CC after 4048	21	Frameshift
Y1307X	T to A at 4053	21	Tyr to Stop at 1307
E1308X	G to T at 4054	21	Glu to Stop at 1308

CF mutations including those known under symbols: 2789+5G>A; 711+1G>T; W1282X; 3120+1G>A; d1507; dF508; (F508C, 1507V, 1506V); N1303K; G542X, G551D, R553X, R560T, 1717-1G>A: R334W, R347P, 1078delT; R117H, I148T, 621+1G>T; G85E; R1162X, 3659delC; 2184delA; A455E, (5T, 7T, 9T); 3849+10kbC>T; and 1898+1G>A, are described in U.S. Patent application serial no. 396,894, filed April 22, 1989, application Serial No. 399,945, filed Aug. 29, 1989, application Serial No. 401,609 filed Aug. 31, 1989. and U.S. Patent Nos. 6,011,588 and 5,981,178, which are hereby incorporated by reference in their entirety. Any and all of these mutations can be detected using nucleic acid amplified with the invention primers as described herein.

[0039] CF mutations in the amplified nucleic acid may be identified in any of a variety of ways well known to those of ordinary skill in the art. For example, if an amplification product is of a characteristic size, the product may be detected by examination of an electrophoretic gel for a band at a precise location. In another embodiment, probe molecules that hybridize to the mutant or wildtype CF sequences can be used for detecting such sequences in the amplified product by solution phase or, more preferably, solid phase hybridization. Solid phase hybridization can be achieved, for example, by attaching the CF probes to a microchip. Probes for detecting CF mutant sequences are well known in the art. See Wall et al. "A 31-mutation assay for cystic fibrosis testing in the clinical molecular diagnostics laboratory," Human Mutation, 1995;5(4):333-8, which specifies probes for CF mutations ΔF508 (exon 1), G542X

(exon 11), G551D (exon 11), R117H (exon 4), W1282X (exon 20), N1303K (exon 21), 3905insT (exon 20), 3849+10Kb (intron 19), G85E (exon 3), R334W (exon 7), A455E (exon 9), 1898+1 (exon 12), 2184delA (exon 13), 711+1 (exon 5), 2789+5 (exon 14b), Y1092x exon 17b), ΔI507 (exon 10), S549R(T-G) (exon 11), 621+1 (exon 4), R1162X (exon 19), 1717-1 (exon 11), 3659delC (exon 19), R560T (exon 11), 3849+4(A-G) (exon 19), Y122X (exon 4), R553X (exon 11), R347P (exon 7), R347H (exon 7), Q493X (exon 10), V520F (exon 10), and S549N (exon 11). Probes for additional CF mutations include those shown in Table 17.

Table 17: Probes for Detection of CF mutations

CF Mutation	Name	Sequence
I148T	SNP1	5'-CCATTTTTGGCCTTCATCACA-3' (SEQ ID NO: 33)
2184delA	SNP3	5'-GATCGATCTGTCTCCTGGACAGAAACAAAA AA-3' (SEQ ID NO: 34)
D1270N	SNP5	5'-GACTGATCGATCGTTATTGAATCCCAAGACAC ACCAT-3' (SEQ ID NO: 35)
3120+1 G->A	SNP6	5'-GACTGATCGATCGATCCCTCTTACCATATTT GACTTCATCCAG-3' (SEQ ID NO: 36)

[0040] CF probes for detecting mutations as described herein may be attached to a solid phase in the form of an array as is well known in the art (see, U.S. Patent 6,403,320 and 6,406,844). For example, the full complement of 24 probes for CF mutations with additional control probes (30 in total) can be conjugated to a silicon chip essentially as described by Jenison et al., Biosens Bioelectron. 16(9-12):757-63 (2001) (see also U.S. Patent No. 6,355,429 and 5,955,377). Amplicons that hybridized to particular probes on the chip can be identified by transformation into molecular thin films. This can be achieved by contacting the chip with an anti-biotin antibody or streptavidin conjugated to an enzyme such as horseradish peroxidase. Following binding of the antibody(or streptavidin)-enzyme conjugate to the chip, and washing away excess unbound conjugate, a substrate can be added such as tetramethylbenzidine (TMB) {3,3',5,5'Tetramethylbenzidine} to achieve localized deposition (at the site of bound antibody) of

a chemical precipitate as a thin film on the surface of the chip. Other enzyme/substrate systems that can be used are well known in the art and include, for example, the enzyme alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate as the substrate. The presence of deposited substrate on the chip at the locations in the array where probes are attached can be read by an optical scanner. U.S. Patent No. 6,355,429 and 5,955,377, which are hereby incorporated by reference in their entirety including all charts and drawings, describe preferred devices for performing the methods of the present invention and their preparation, and describes methods for using them.

[0041] The binding of amplified nucleic acid to the probes on the solid phase following hybridization may be measured by methods well known in the art including, for example, optical detection methods described in U.S. Patent No. 6,355,429. In preferred embodiments, an array platform (see, e.g., U.S. Patent 6,288,220) can be used to perform the methods of the present invention, so that multiple mutant DNA sequences can be screened simultaneously. The array is preferably made of silicon, but can be other substances such as glass, metals, or other suitable material, to which one or more capture probes are attached. In preferred embodiments, at least one capture probe for each possible amplified product is attached to an array. Preferably an array contains 10, more preferably 20, even more preferably 30, and most preferably at least 60 different capture probes covalently attached to the array, each capture probe hybridizing to a different CF mutant sequence. Nucleic acid probes useful as positive and negative controls also may be included on the solid phase or used as controls for solution phase hybridization.

[0042] In still another approach, wildtype or mutant CF sequence in amplified DNA may be detected by direct sequence analysis of the amplified products. A variety of methods can be used for direct sequence analysis as is well known in the art. See, e.g., The PCR Technique: DNA Sequencing (eds. James Ellingboe and Ulf Gyllensten) Biotechniques Press, 1992; see also "SCAIP" (single condition amplification/internal primer) sequencing, by Flanigan et al. Am J Hum Genet. 2003 Apr;72(4):931-9. Epub 2003 Mar 11.

[0043] In yet another approach for detecting wildtype or mutant CF sequences in amplified DNA is single nucleotide primer extension or "SNuPE." SNuPE can be performed as described in U.S. Pat. No. 5,888,819 to Goelet et al., U.S. Pat. No. 5,846,710 to Bajaj, Piggee, C. et al. Journal of Chromatography A 781 (1997), p. 367-375 ("Capillary Electrophoresis for the Detection of Known Point Mutations by Single-Nucleotide Primer Extension and Laser-Induced

Fluorescence Detection"); Hoogendoorn, B. et al., Human Genetics (1999) 104:89-93, ("Genotyping Single Nucleotide Polymorphism by Primer Extension and High Performance Liquid Chromatography"); and U.S. Pat. No. 5,885,775 to Haff et al. (analysis of single nucleotide polymorphism analysis by mass spectrometry). In SNuPE, one may use as primers such as those specified in Table 17.

[0044] Still another approach for detecting wildtype or mutant CF sequences in amplified DNA is oligonucleotide ligation assay or "OLA". The OLA uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target molecules. One of the oligonucleotides is biotinylated, and the other is detectably labeled. If the precise complementary sequence is found in a target molecule, the oligonucleotides will hybridize such that their termini abut, and create a ligation substrate that can be captured and detected. See e.g., Nickerson et al. (1990) Proc. Natl. Acad. Sci. U.S.A. 87:8923-8927, Landegren, U. et al. (1988) Science 241:1077-1080 and U.S. Pat. No. 4,998,617.

[0045] These above approaches for detecting wildtype or mutant CF sequence in the amplified nucleic acid is not meant to be limiting, and those of skill in the art will understand that numerous methods are known for determining the presence or absence of a particular nucleic acid amplification product.

[0046] In another aspect the present invention provides kits for one of the methods described herein. In various embodiments, the kits contain one or more of the invention primers in an amount suitable for amplifying a specified CFTR sequence from at least one nucleic acid containing sample. The kit optionally contain buffers, enzymes, and reagents for amplifying the CFTR nucleic acid via primer-directed amplification. The kit also may include one or more devices for detecting the presence or absence of particular mutant CF sequences in the amplified nucleic acid. Such devices may include one or more probes that hybridize to a mutant CF nucleic acid sequence, which preferably is attached to a bio-chip device, such as any of those described in U.S. Patent No. 6,355,429. The bio-chip device optionally has at least one capture probe attached to a surface on the bio-chip that hybridizes to a mutant CF sequence. In preferred embodiments the bio-chip contains multiple probes, and most preferably contains at least one probe for a mutant CF sequence which, if present, would be amplified by a set of flanking primers. For example, if five pairs of flanking primers are used for amplification, the device

would contain at least one CF mutant probe for each amplified product, or at least five probes. The kit also preferably contains instructions for using the components of the kit.

[0047] The following examples serve to illustrate the present invention. These examples are in no way intended to limit the scope of the invention.

Example 1 – Detection of CF mutations from whole blood

A. Extraction of DNA

[0048] Suitable samples may include fresh tissue, e.g., obtained from clinical swabs from a region where cells are collected by soft abrasion (e.g., buccal, cervical, vaginal, etc. surfaces) or biopsy specimens; cells obtained by amniocentesis or chorionic villus sampling; cultured cells, or blood cells; or may include fixed or frozen tissues. The following example describes preparation of nucleic acids from blood.

[0049] 50 µL of whole blood was mixed with 0.5 ml of TE (10 mM Tris HCl, 1 mM EDTA, pH 7.5) in a 1.5 mL microfuge tube. The sample was spun for 10 seconds at 13,000 x g. The pellet was resuspended in 0.1 mL of TE buffer with vortexing, and pelleted again. This procedure was repeated twice more, and then the final cell pellet was resuspended in 100 µl of K buffer 50 mM KCl, 10 mM Tris HCl, 2.5 mM $MgCl_2$, 0.5% Tween 20, 100 µg/mL proteinase K, pH 8.3) and incubated 45 minutes at 56 °C, then 10 minutes at 95 °C to inactivate the protease.

B. Amplification from DNA

[0050] Individual amplifications were prepared in a volume of 13.5 µl, which was added to 96 well microtiter plates. Each amplification volume contained 2 µl of the DNA sample (generally 10-100 ng of DNA), 11.5 µl of PCR-Enzyme Mix (PCR-Enzyme mix stock was prepared with 11.3 µl master mix, 0.25 µl $MgCl_2$ (from 25 mM stock), and 0.2 µl of FasStar Taq (source for last two reagents was Roche Applied science, Cat. No. 2 032 937). Master mix contained 5'biotinylated primers, Roche PCR buffer with $MgCl_2$, Roche GC rich solution (cat. No. 2 032 937), bovine serum albumin (BSA) (New England BioLabs, Cat no. B9001B), and NTPs (Amersham Biosciences, Cat no. 27-2032-01).

[0051] The final concentration in the PCR for $MgCl_2$ was 2.859 mM, for BSA was 0.725 µg/µl, and for each dNTP was 0.362 mM. Primer final concentrations of biotinylated primers were 0.29 µM for each of SEQ ID NOs: 9, 10, 13 and 14 (exon 12 and 21), 0.145 µM for each of

SEQ ID NOs: 3-6 (exons 4 and i19), 0.091 μM for each of SEQ ID NOs: 7, 8, 15, 16, and 29-32 (exons 19, 7, i5 and 11), 0.072 μM for each of SEQ ID NOs: 11, 12, 19 and 20 (exon 3 and 14), 0.060 μM for each of SEQ ID NOs: 17, 18 and 23-28 (exons 16, 20, 13 and 10), and 0.036 μM for each of SEQ ID NOs: 21 and 22, (exon 9).

[0052] PCR was conducted using the following temperature profile: step 1: 96 °C for 15 minutes; step 2: 94 °C for 15 seconds; step 3: decrease at 0.5 °C/second to 56 °C; step 4: 56 °C for 20 seconds; step 5: increase at 0.3 °C/second to 72°C, step 6: 72 °C for 30 seconds; step 7: increase 0.5 °C up to 94 °C; step 8: repeat steps 2 to 7 thirty three times; step 9: 72 °C for 5 minutes; step 10: 4 °C hold (to stop the reaction).

C. Detection of CF amplicons

[0053] The presence of CF sequences in the amplicons was determined by hybridizing the amplified product to a solid phase strip containing an array of 50 probes specific for CF mutations and CF wildtype sequence (LINEAR ARRAY CF GOLD 1.0™, Roche Diagnostics) in accordance with the manufacturer's instructions. Detection of hybridized amplicons was by streptavidin-HRP conjugate and development using the TMB as substrate.

[0054] The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other physical and electronic documents.

[0055] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and

variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0056] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0057] Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.